Journal of Receptors and Signal Transduction

ISSN: 1079-9893 (Print) 1532-4281 (Online) Journal homepage: https://www.tandfonline.com/loi/irst20

Resveratrol diminishes bisphenol A-induced oxidative stress through TRPM2 channel in the mouse kidney cortical collecting duct cells

Bilal Çiğ & Kenan Yildizhan

To cite this article: Bilal Çiğ & Kenan Yildizhan (2020) Resveratrol diminishes bisphenol A-induced oxidative stress through TRPM2 channel in the mouse kidney cortical collecting duct cells, Journal of Receptors and Signal Transduction, 40:6, 570-583, DOI: 10.1080/10799893.2020.1769657

To link to this article: https://doi.org/10.1080/10799893.2020.1769657

Published online: 09 Jun 2020.

JOURNAL OF RECEPTORS AND SIGNAL TRANSDUCTION
2020, VOL. 40, NO. 6, 570–583
https://doi.org/10.1080/10799893.2020.1769657

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Resveratrol diminishes bisphenol A-induced oxidative stress through TRPM2 channel in the mouse kidney cortical collecting duct cells

Bilal Çiğ[a] and Kenan Yildizhan[b]

[a]Department of Physiology, Faculty of Medicine, Ahi Evran University, Kirsehir, Turkey; [b]Department of Biophysics, Faculty of Medicine, Van Yuzuncu Yil University, Van, Turkey

ABSTRACT

Bisphenol A (BisPH-A) is a latent danger that threatens our health, which we frequently exposure in our modern life (e.g. the widespread use of drinking water in plastic pet bottles). But the BisPH-A induced transient receptor potential melastatin 2 (TRPM2)-mediated oxidative stress and apoptosis in these cells has not been studied yet. Calcium (Ca^{2+}) plays an important role in a versatile intracellular signal transduction that works over a wide range to regulate oxidative stress processes. TRPM2 is activated by oxidative stress and it has emerged as an important Ca^{2+} signaling mechanism in a variety of cells, contributing many cellular functions including cell death. Resveratrol (RESV), which belongs to the polyphenol group, acts as an antioxidant, eliminating cellular oxidative stress and increasing the body's resistance to diseases. The current study aimed to elucidate the effect of antioxidant resveratrol on TRPM2-mediated oxidative stress induced by BisPH-A exposure in the mouse kidney cortical collecting duct cells ($mpkCCD_{cl4}$). The cells were divided into four groups as control, resveratrol (50 μM for 24 h), BisPH-A (100 μM for 24 h) and BisPH-A + RESV. Intracellular free Ca^{2+} concentrations and TRPM2 channel currents were high in BisPH-A treated cells, but decreased with resveratrol treatment. In addition, BisPH-A induced mitochondrial membrane depolarization, reactive oxygen species (ROS), caspase 3, caspase 9 and apoptosis values were decreased by the resveratrol treatment. In conclusion, resveratrol protected cells from BisPH-A induced oxidative damage. In this study, we showed that TRPM2 channel mediates this protective effect of resveratrol.

ARTICLE HISTORY
Received 19 January 2020
Revised 11 May 2020
Accepted 11 May 2020

KEYWORDS
Bisphenol A; Oxidative stress; Apoptosis; TRPM2; Resveratrol

Introduction

Bisphenol A (BisPH-A) is a chemical substance in phenol structure and it is used in hardening of plastic material especially in industry. In a first study in 1936, it was found that BisPH-A showed estrogenic activity in overectomized rats [1]. Although BisPH-A did not come into use as a synthetic estrogen preparation, it was first used in the 1940s for the purpose of making epoxy resins for commercial purposes [2]. Nowadays, it is included in plastic food containers, baby bottles, adhesives, paints and tooth fillers [3,4]. This chemical substance, which is produced millions of tons every year, is found in many man-made materials such as electronic devices, baby toys and thermal receipt papers [5]. In particular, the use of BisPH-A in containers where canned foods are stored for a long time, increases the risk of human exposure to this chemical [6]. There are also studies indicating that even respiratory and dermal contact may cause BisPH-A exposure [7]. BisPH-A may also be effective on different endocrine gland functions, known as endocrine disruptors, ranging from the synthesis of the hormone to its metabolism and binding to the receptor [8,9]. In addition, there are several studies related to linking exposure to BisPH-A with health problems such as cardiovascular diseases, diabetes, thyroid dysfunction, obesity and reproductive disorders [9,10]. Besides, it was reported that there is a relationship between BisPH-A level and some types of cancer for instance; thyroid, prostate and lung cancer [11].

Although many studies have been conducted on BisPH-A, its mechanisms of action are still poorly known [12]. Studies have shown that exposure to toxic BisPH-A disrupts intracellular oxidant/antioxidant balance due to increased free oxygen radicals (ROS) in the environment [13]. *In vitro* studies revealed that cells exposed to BisPH-A were dragged into apoptosis due to excessive ROS production and mithocondrial disorder [14]. Mitochondrial dysfunction and increased production of ROS lead to excessive intracellular calcium increase $[Ca^{2+}]_i$ which has proven to play an important role in both cell survival and death [15]. Disruption of intracellular Ca^{2+} concentration may trigger apoptosis, and cell death pathways, causing dysfunction of proteins and disruption of ion flow [16]. Apoptotic findings and increasing ROS and Ca^{2+} in intracellular fluid during early necrotic process support this idea [17]. Current studies have shown that resveratrol with high antioxidant properties is beneficial against ROS-induced apoptotic cell death [18].

The resveratrol is found extensively in red grape peel synthesized in grapes against biotic and abiotic stress conditions [19]. It is emphasized that resveratrol has antifungal, antimicrobial, antiinflammatory, antitumor and antioxidant

CONTACT Bilal Çiğ bilalcig@gmail.com Department of Physiology, Faculty of Medicine, Ahi Evran University, Kirsehir 40100, Turkey

effects in the literatüre [20]. Here, based on its antioxidant properties, we wanted to investigate the role of RESV against Bisph-A-induced oxidative stress and apoptosis.

The TRP superfamily are calcium permeable nonselective cation channels that are activated by mechanical [21], thermal, and chemical stimulants and play a vital role in many physiological events, particularly cell proliferation, oxidative stress, cytokine production and cell death [22]. TRPM2 (melastatin 2) from the TRP subfamily is activated by adenosine disphosphoribose (ADPR), Ca^{2+}, ROS, and reactive nitrogen species and inhibited by protons (acidic PH), ACA, 2APB, and adenosine monophosphate. TRPM2 channels have an important functions in immune and inflammatory responses [23].

Water in plastic bottles containing BisPH-A, which we consume too much every day, touches the cells in the cortical collection ducts in our kidneys. Therefore, to investigate this condition *in vitro*, we used renal cortical collection duct cell line ($mpkCCD_{cl4}$) obtained from mouse kidneys. Although many oxidative stress parameters have been studied in this cell line to date, oxidative stress mediated by TRPM2 cation channels of BisPH-A and consequent apoptosis has not yet been studied. In addition, this study aimed to explain the effect of antioxidant resveratrol on TRPM2-induced oxidative stress induced by BisPH-A exposure in $mpkCCD_{cl4}$ cells.

Material and Methods

Reagents

Bisphenol-A (BisPH-A), Resveratrol (RESV), dimethyl sulfoxide (DMSO), L-glutamine, penicillin/streptomycin, Trypsin-EDTA, 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide dye, hydrogen peroxide (H_2O_2), ADP-ribose (ADPR), Dihydrorhodamine 123 (DHR 123) were purchased from Sigma Aldrich, St. Louis, MO, USA. N-(p-Amylcinnamoyl) Anthranilic Acid (ACA) as a TRPM2 blocker was purchased from Santa Cruz (Istanbul, Turkey). Hoechst, Annexine V FITCH (aV)/propidium iodide (PI) were purchased from Cell Signaling (Istanbul, Turkey).

Cell culture

The mouse renal cortical collecting duct cells ($mpkCCD_{cl4}$) were provided by Prof. Dr. Franziska Theilig at Fribourg University, Germany. The $mpkCCD_{cl4}$ cells were maintained as described before [24]. Cells were prepared for electrophysiological recordings, calcium signal, confocal, apoptosis analysis and western-blott analysis. Cells were replicated in curved neck 25cm^2 flasks. They were attached to 35mm coverslips (Mattek Corporation Inc., USA) for confocal analysis.

Groups

The $mpkCCD_{cl4}$ cells were divided into four groups as follows: (G-1) **Control** Group, these cells were kept in cell culture conditions for 24 h without incubating with BisPH-A and RESV (in 25-cm^2 culture flasks at 37 °C in 5% CO2 95% air incubator); (G-2) **RESV** group, these cells were incubated with RESV (50 μM) for 24 h [25]; (G-3) **BisPH-A** group, these cells were incubated with BisPH-A (100 μM) for 24 h as described in a previous study [26]; and (G-4) **BisPH-A+RESV** group in which cells were incubated with BisPH-A (100 μM) and RESV (50 μM) for 24 h.

Obtaining electrophysiological recordings from a single cell

Electrophysiological recordings were obtained on mpkCCDcl4 cells (HEKA Patch-clamp set, Germany, whole-cell configuration, room temperature 22 ± 2 °C). Details of the used tampons were prepared according to the previous studies [27]. Since TRPM2 is activated in high calcium, about 10 times the intracellular calcium concentration was used here (1 μM). The voltage-clamp technique was used here and the holding potential was -60 mV. Current-Voltage graphs were obtained (Voltage ramps were adjusted between -150, +150 mV, over 200 ms). ADPR was administered in-pipette (1mM), while ACA (TRPM2 antagonist, 25 μM) was extracellular. In the recordings obtained from a cell, current amplitudes were divided to capacitance and current density was obtained (pA/pF).

Intracellular Free Calcium Concentration ($[Ca^{2+}]_i$)

Fura 2-AM calcium indicator, which specifically binds to the calcium inside the cell, was used to determine the intracellular calcium concentration [22]. Trypsin-treated cells were treated with 5 μM fura2-AM (Calbiochem, Darmstadt, Germany) in a 37 oC shaking water bath for 45 minutes after washing and centrifugation. The cell suspension (containing at least 1 million cell) treated with Fura 2-AM, a fluorescent marker based on ratiometric measurement, was placed in a 37 C transparent cuvette with magnetic stirrer in the Carry Eclipse spectrofluorometer (Varian Inc, Australia). Emission values of 505 nm were recorded at 340/380 nm excitations. Calcium amounts in cells were calculated according to Grynkiewicz method [28,29]. $MpkCCD_{cl4}$ cells were stimulated with H_2O_2 (100 μM). TRPM2 channel activations were inhibited by ACA (25 μM).

Determination of intracellular Ca^{2+} fluorescence intensity by confocal microscopy

The Ca^{2+} density in $mpkCCD_{cl4}$ cells was determined using a zeiss immersion objective 40xoil laser confocal microscopy (LSM-800, Zeiss, Germany) with a fluo3 calcium marker (Calbiochem, Germany). H_2O_2 (1mM) and ACA (25 μM) (agonist and antagonist, respectively) were used to determine TRPM2 channel activation by confocal microscopy. The average fluorescence intensity (fluo-labeled calcium) was expressed according to previous studies [24].

Assay of cell viability

Cell viability was analyzed with colorimetric tetrazolium bromide (MTT) [30]. Cells were treated with 0.5 mg / ml MTT

at 37 ° C for 1,5 hours. It was analyzed at appropriate absorbance values (490 nm) in the multi-well reader (Infinite pro200; Tecan Austria). The data were plotted the fold increase.

Assay of apoptosis levels, caspase-3 and caspase-9 activities

As previously described [31], it was analyzed in a multi-well reader (Infinite pro200; Tecan Austria) using the ready kit for apoptosis analysis (Apopercentage kit, Biocolor, Northern Ireland). Apoptotic cells were analyzed in plate reader at 550 nm after apopercentage dye releaze treatment. Caspase-3 and caspase-9 analyzes were performed according to the previously reported method [32]. Cleavage of caspase 3 and caspase 9 substrates (Bubendorf, Switzerland) was analyzed at excitation and amission wavelengths of 360 and 460 nm, respectively (Infinite pro200).

Determination of mitochondrial membrane potential ($\Delta\Psi m$)

JC-1 fluorescent marker (Thermo Fisher, Istanbul, Turkey) was used for the determination of mitochondrial membrane depolarization. The oligomer (J-aggregate) formed in mitochondria, whose membrane is depolarized, emits green fluorescence at 530 nm and red at 590 nm. The cells treated with JC-1 were analyzed in the multi-well plate reader at the excitation and emission wavelengths of 485 and 530 nm for the green signal, 540 and 590 nm for the red signal, respectively [33].

Determination of reactive oxygen species (ROS) production

DHR123 was used to determine ROS production [34]. DHR is normally in insoluble form, in this process it is converted to rhodamine 123 and determines the amount of ROS. As previously described, cells were treated with DHR123 for 25 minutes at 37 °C [35]. The amount of Rhodamine 123 used in the determination of ROS was analyzed in multiple well reader at 488 nm excitation and 543 nm emission wave lengths.

Western blot analyses

Caspase 3, caspase 9, beta actin (polyclonal antibody) Poly-ADPR polymerase 1 (PARP-1) (polyclonal antibody) secondary antibodies were used (GE Healthcare, UK). The analysis steps were performed as previously described [36]. Here, we analyzed how much the levels of caspase, PARP-1 protein expression changed, with the effect of BisPH-A and RESV. Rabbit anti-p-actin (1: 2000) was used as a control.

Imaging ROS generation and JC-1 by Laser Confocal Microscope Analysis

MpkCCD$_{c14}$ cells were treated with 1 μM DHR123 for 20 min and than intracellular ROS production analyzed under a laser confocal microscope [37]. Cells were treated with JC-1 5 μl 15 min 37 ° C to determine the mitochondrial membrane depolarization. ROS and JC-1 measurements in the cells were analyzed under a laser confocal microscope by stimulating at a wavelength of 488 nm[38].

Annexin V-FITC and Propidium Iodide (PI) Assays by Laser Confocal Microscope

Annexin V-FITCH (1 μl), PI (10 μl) (Santa Cruz Biotechnology, Inc. Texas USA) was used for the determination of BisPH-A induced apoptosis and analyzed under a laser confocal microscope (LSM-800) [39]. The fluorescence intensity in the cells was measured using the ZEN program and analyzed with Image J / Imaris software [37].

Bradford Protein Assay for the Quantification of Total Protein

Enzyme assays were adjusted for variations in cell number using the Bradford protein assay described by Bradford [24].

Statistical analyses

SPSS program was used in 18.0 (IBM, New York, NY, USA) to calculate statistical significance. Fisher's smallest difference (LSD) test was used. Statistical significance was determined by nonparametric test (Mann-Whitney U) ($p \leq 0.05$). Results are expressed as mean ± standard deviation (SD).

Results

Effects of BisPH-A and RESV on TRPM2 currents in the mpkCCD$_{cl4}$ cells

Results of current density reported as pA/pF in the patch-clamp records are shown in Figure 1. There were no significant currents in the absence of the TRPM2 agonists and antagonists (ADPR and ACA) (Figure 1(a)). The TRPM2 channel in the patch-clamp experiments was gated in the mpkCCD$_{cl4}$ cells by intracellular ADPR, although they were reversibly blocked by ACA and $NMDG^+$ (replacement of Na^+). As expected, the current densities in the mpkCCD$_{cl4}$ cells were increased to about 200 pA/pF in the Control + ADPR group compared with the Control group (5 pA/pF) alone ($p \leq 0.001$) (Figure 1(a,b,f)). However, the current density of TRPM2 was about 8-fold ($p \leq 0.001$) lower in the Control + ADPR + ACA group compared to the Control + ADPR group (Figure 1(b,f)). We observed about 2-fold increase in the current densities in mpkCCD$_{cl4}$ cells in the BisPH-A group compared to Control + ADPR group (Figure 1(d,f)). The TRPM2 currents decreased about 10-fold in the BisPH-A + ACA group as compared to the BisPH-A

group (Figure 1(d,f)). In addition to over-activating the TRPM2 channel by BisPH-A, when RESV was applied with BisPH-A, there was a statistically significant reduction in the activation of these channels (Figure 1(d,e,f)). These results clearly indicate that BisPH-A induces Ca^{2+} influx through TRPM2 activation and BisPH-A induced TRPM2 currents were significantly decreased by treatment with RESV. ($^{a}p \leq 0.001$ vs control; $^{b}p \leq 0.001$ vs control + ADPR; $^{c}p \leq 0.001$ versus control, control + ADPR; $^{d}p \leq 0.001$ and $^{e}p \leq 0.001$ vs BisPH-A; $^{f}p \leq 0.001$ vs BisPH-A + RESV).

BisPH-A-induced TRPM2 channel overactivity in mpkCCDc14 cells: based on calcium signal results

In this study, we aimed to investigate whether RESV therapy may affect H2O2-induced Ca^{2+} mobilization in mpkCCDc14 cells exposed to BisPH-A. We used ACA as an antagonist. Figure 2(a,b) represent the intracellular calcium concentration change $[Ca^{2+}]_i$ between the groups as column and curve graphs, respectively. The change due to TRPM2 channel activation in the BisPH-A group $[Ca^{2+}]_i$ was significantly higher than the control group (bp <0.001). ACA and RESV strongly neutralized the BisPH-A effect by inhibiting TRPM2 channels (cp $\leq$ 0.001 and dp $\leq$ 0.001 versus BisPH-A group).

Effects of BisPH-A and RESV on florescence intensity of Ca^{2+} through TRPM2 activation in the mpkCCD$_{cl4}$ cells

Here we wanted to clarify the role of TRPM2 inhibitor (ACA) on free Ca^{2+} fluorescent intensity in mpkCCD$_{c14}$ cells. Treatment of cells with ACA and RESV suppressed the Ca^{2+} fluorescence intensity caused by BispH-A detected by laser confocal microscopy (LSM 800, Zeiss, Ankara, Turkey) analysis (Figure 3(a,b); $^{e}p \leq 0.001$ vs Control; $^{g}p \leq 0.001$, vs BisPH-A groups). These results confirm the patch-clamp and calcium signal results. RESV completely inhibited BispH-A from over-activating the oxidative stress-mediated TRPM2 channel. In therapeutic approaches to BisPH-A, it is important to consider the TRPM2 gate, a ROS sensor.

RESV decreased mitochondrial membrane depolarization and ROS in mpkCCD$_{cl4}$ cells

It has been reported that Ca^{2+} induced by BisPH-A triggers membrane depolarization in mitochondria [40]. This triggers reactive oxygen species to increase in the environment. Here, we investigated BispH-A induced mitochondrial membrane depolarization and ROS increase in mpkCCD$_{c14}$ cells under laser confocal microscope (LSM 800, Zeiss, Ankara, Turkey) using markers JC-1 and DHR-123. Resveratrol (RESV) treatment prevents the increase in these values. While BisPH-A significantly increased ROS and JC-1 values compared to Control, RESV significantly suppressed these effects ($^{*}p \leq 0.001$ vs. control groups, $\#p \leq 0.001$ vs. BisPH-A groups.). This can be attributed to the ROS scavenging effect of RESV.

RESV suppressed BisPH-A-induced apoptosis in mpkCCD$_{cl4}$ cells

Oxidative phosphorylation center mitochondria are also the main source of ROS production. However, excessive accumulation of mitochondrial calcium causes an increase in the electron transport system and then mitochondrial membrane depolarization occurs [41]. Increased mitochondrial membrane depolarization causes the release of cytochrome C from the electron transport system to cytosol. This is the starting signal of the apoptotic cascades. After observing activation in the mitochondrial ROS and TRPM2 channel, we suspected whether cell death was increased in mpkCCD$_{c14}$ cells. Propidium iodide (PI) is an indicator of dead cells, while Hoechst dye is an indicator of living cells (Figure 4). The percentage of dead cells increased with BisPH-A exposure. The increase in cell death was suppressed by RESV therapy. Therefore, we observed the inhibitory effect of RESV on cell death in mpkCCD$_{c14}$ cells.

Annexin V and PI Results

In addition to the Hoechst-PI results above, Annexin V and PI fluorescence intensity levels were evaluated in this section and their arbitrary units are shown in Figure 5. Annexin V is commonly used to detect apoptotic cells by binding to an apoptosis marker, phosphatidylserine, on the outer leaf of the plasma membrane [42]. After exposure to BisPH-A, Annexin V and PI levels in mpkCCD$_{c14}$ cells increased significantly ($p \leq 0.001$), but after BisPH-A exposure, fluorescent intensities in the RESV-treated group were significantly lower than the BisPH-A group.

PARP-1, caspase-3 and caspase-9 expression levels in mpkCCD$_{cl4}$

The expression levels of PARP-1, caspase-3 and caspase-9 in the mpkCCDc$_{14}$ cells of the four groups are shown in Figure 6(a,b), respectively. The PARP1, caspase-3 and caspase-9 expression levels of the mpkCCD$_{c14}$ cells were significantly increased by BisPH-A exposed ($^{*}p \leq 0.001$), but their expression levels were decreased with RESV treatments ($^{\#}p \leq 0.001$).

Apoptosis, caspases, ROS, JC-1 and cell viability (MTT) results

Compared to the control, in the BisPH-A exposed mpkCCD$_{c14}$ cells, increased the level of apoptosis (approximately 1.6-fold, $^{b}p \leq 0.001$ vs control) (Figure 6(c)), but significantly reduced cell viability levels (approximately 4-fold, $^{c}p \leq 0.001$ vs control) (Figure 6(h)). RESV treatment resulted in a decreased in the level of apoptosis in these cells (approximately 2-fold, $^{a}p \leq 0.001$, Figure 6(c)). In addition, RESV treatment of bisPH-A-exposed cells also significantly reduced apoptosis levels (approximately 2-fold, $^{a}p \leq 0.001$, Figure 6(c)). When the cell viability results were examined, it was observed that TRPM2 blocker (ACA) had a positive contribution compared to control ($^{a}p \leq 0.05$ vs control, Figure 6(h)). The positive effect of

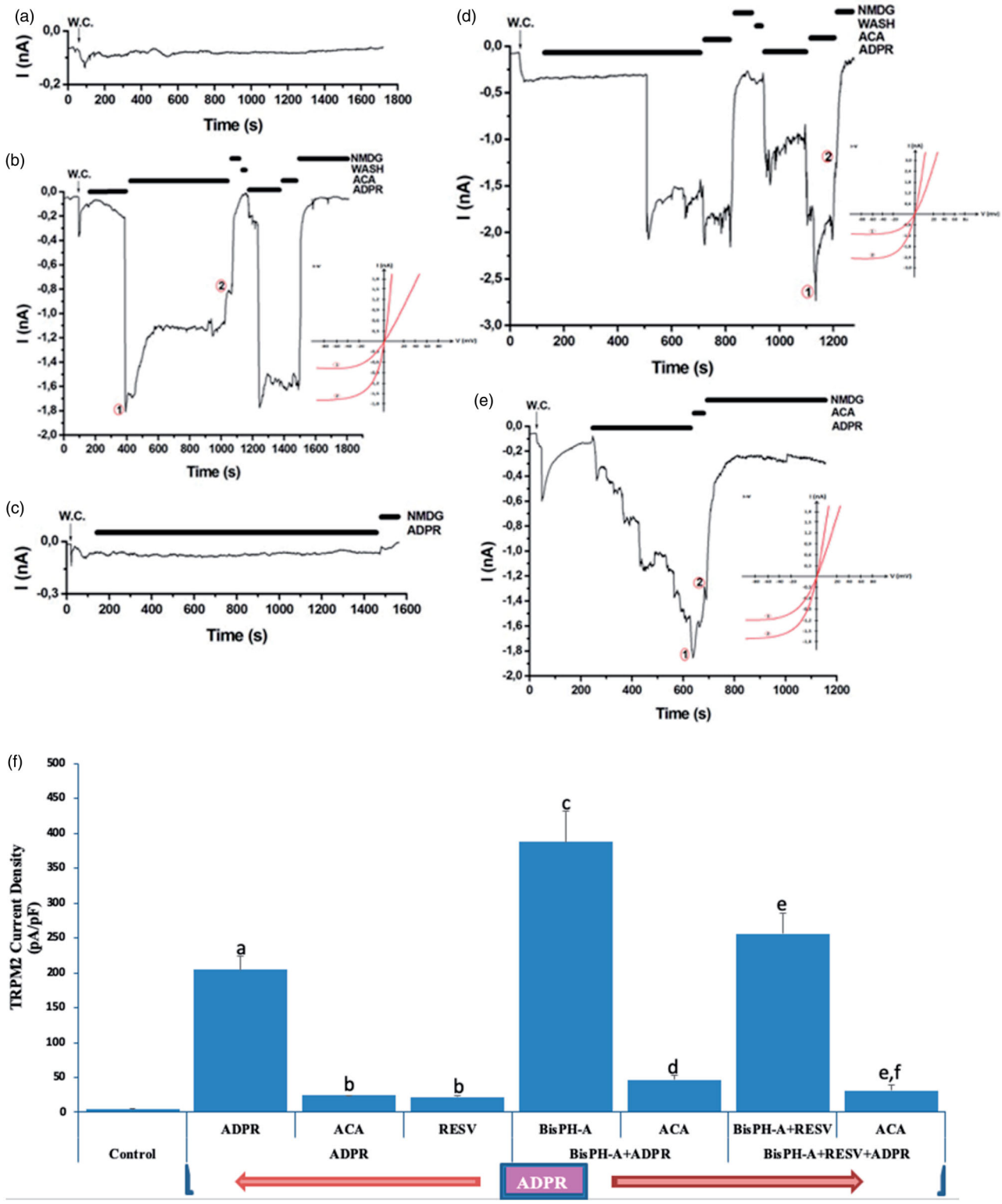

Figure 1. Effects of BisPH-A and Resveratrol (RESV) on TRPM2 channel activity in mpkCCD$_{cl4}$ cells. The holding potential was 60 mV. (a) Control ($n = 10$): Original recordings from control group mpkCCD$_{cl4}$ cell. (b) Control-ADPR group ($n = 10$): TRPM2 currents in the cells were stimulated by ADPR (1 mM) in patch pipette and they were inhibited by ACA (0.025 mM) in the bath. (c) Resveratrol (RESV) group ($n = 10$) TRPM2 currents in the cells were stimulated by ADPR (1 mM) in the patch pipette. (d) BisPH-A group + ADPR ($n = 10$) TRPM2 currents in the cells were stimulated by ADPR (1 mM) in the patch pipette and they were inhibited by ACA (0.025 mM) in the bath. (e) BisPH-A + RESV group ($n = 10$). (f) TRPM2 current density column graph ($^{a}p \leq 0.001$ vs control; $^{b}p \leq 0.001$ vs control + ADPR; $^{c}p \leq 0.001$ versus control, control + ADPR; $^{d}p \leq 0.001$ and $^{e}p \leq 0.001$ vs BisPH-A; $^{f}p \leq 0.001$ vs BisPH-A + RESV). I-V Current voltage relationships of whole-cell currents in the presence of various extracellular cations, activators and inhibitors as indicated. The N-methyl-D-glucamine ($NMDG^{+}$) time is shown where the normal bath solution (140 nM Na^{+}) was exchanged to a solution with $NMDG^{+}$ as main cation (150 mM, no Ca^{2+} present). W.C.: Whole cell.

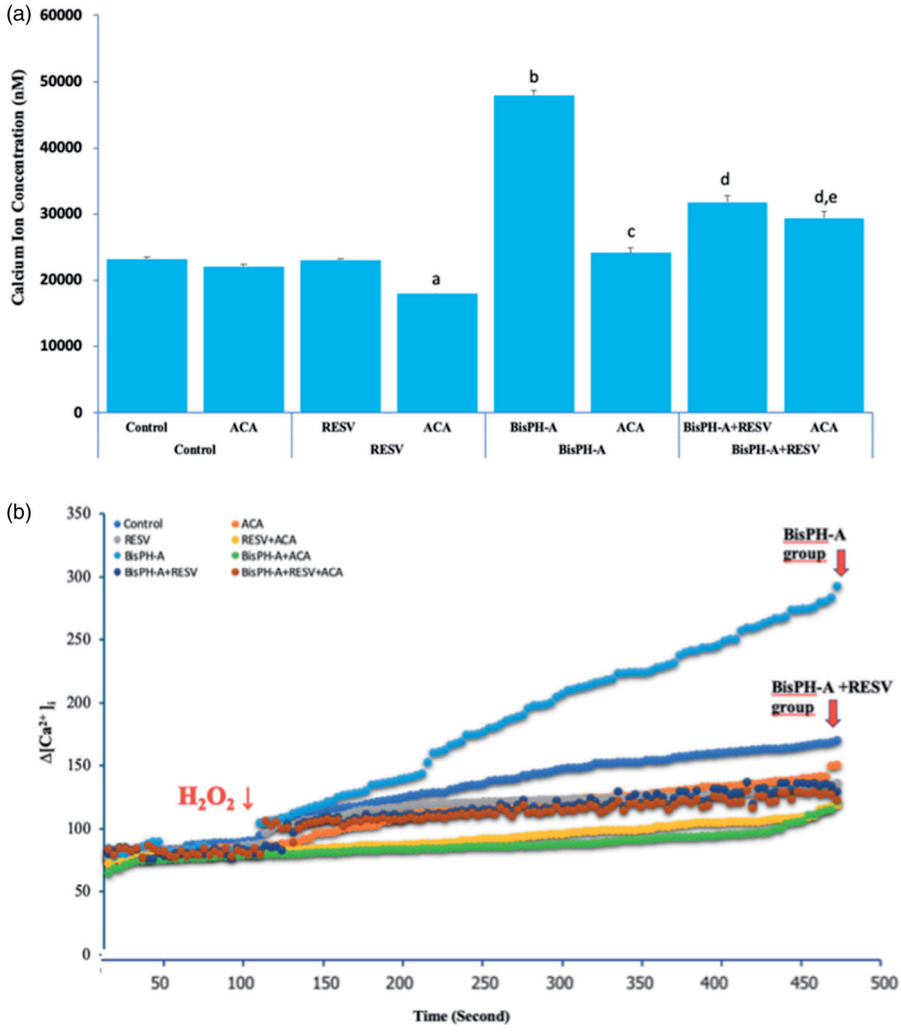

Figure 2. Effect of Resveratrol (RESV) treatment on $[Ca^{2+}]_i$ concentration (a) and calcium influx (b) through block of TRPM2 gate in the mouse kidney cortical collecting duct cells ($mpkCCD_{cl4}$) of control and BisPH-A groups (mean ± SD and $n = 10$). These $mpkCCD_{cl4}$ cells of control, BisPH-A, RESV and BisPH-A + RESV groups were further treated ACA (0.025 mM) before loading Fura-2 for 30 min. The cells in the cuvette are stimulated with H_2O_2 (100 μM) for activate of TRPM2 channels approximately 500 s. ($^{a}p \leq 0.05$ and $^{b}p \leq 0.001$ versus control group. $^{c}p \leq 0.001$ and $^{d}p \leq 0.001$ versus BisPH-A group. $^{e}p \leq 0.05$ versus BisPH-A + RESV group).

ACA (TRPM2 blocker) in terms of cell viability was increased when applied together with RESV ($^{b}p \leq 0.01$ vs RESV, Figure 6(h)). RESV treatment was not effective in cells alone after bisPH-A exposure, but it was a positive contribution to cell viability when administered with ACA. ($^{d}p \leq 0.05$ vs BisPH-A;, $^{e}p \leq 0.05$ vs BisPH-A + RESV, Figure 6(h)). When we look at our ROS and mitochondrial membrane potential results, BisPH-A increased these values compared to control, and RESV treatment improved this situation ($p \leq 0.001$). It is well known that activation of caspase-9 and caspase-3, and mitochondrial membrane depolarization due to intracellular excess Ca^{2+} entry stimulate mitochondrial cell death signals (Bcl2-associated death supporter (Bad), cytochrome c etc.) [27]. Here, in our caspase-3 and caspase-9 results, BisPH-A group was higher compared to control, and RESV restored these values to normal ($p \leq 0.001$).

Discussion

In this study, the role of TRPM2 was investigated in the presence of bisphenol A (BisPH-A) induced oxidative stress in mouse kidney cortical collection duct ($mpkCCD_{cl4}$) cells. These cells are located in the distal nephron, where fluid excretion from the glomerular system is performed, in the cortex of the kidney, where urine is collected after the distal tubule. (Figure 7). We used resveratrol (RESV), which is a strong antioxidant in terms of its protective effect and is a kind of flavonoid that is abundant in grape peel and seed. In this study, we modeled *in vitro* what effects BisPH-A is involved in urine. Human exposure to BisPH-A is a worldwide concern. A recent study found that more than 90% of North Americans had detectable urinary BisPH-A [43,44]. BisPH-A, whose commercial use has become widespread since the second half of the twentieth century, is used in the production of polycarbonate plastic and epoxy resins. The main reason that BisPH-A is preferred in such a wide area is that it gives the materials harder, durable and impermeable properties. As an example, BisPH-A is included in many materials such as water bottles, sports equipment, interior coatings of industrial food and beverage packaging, baby bottles, dental materials, detergents, thermal papers given in shopping vouchers, which are indispensable for daily life. It is noteworthy that some famous companies have started to write

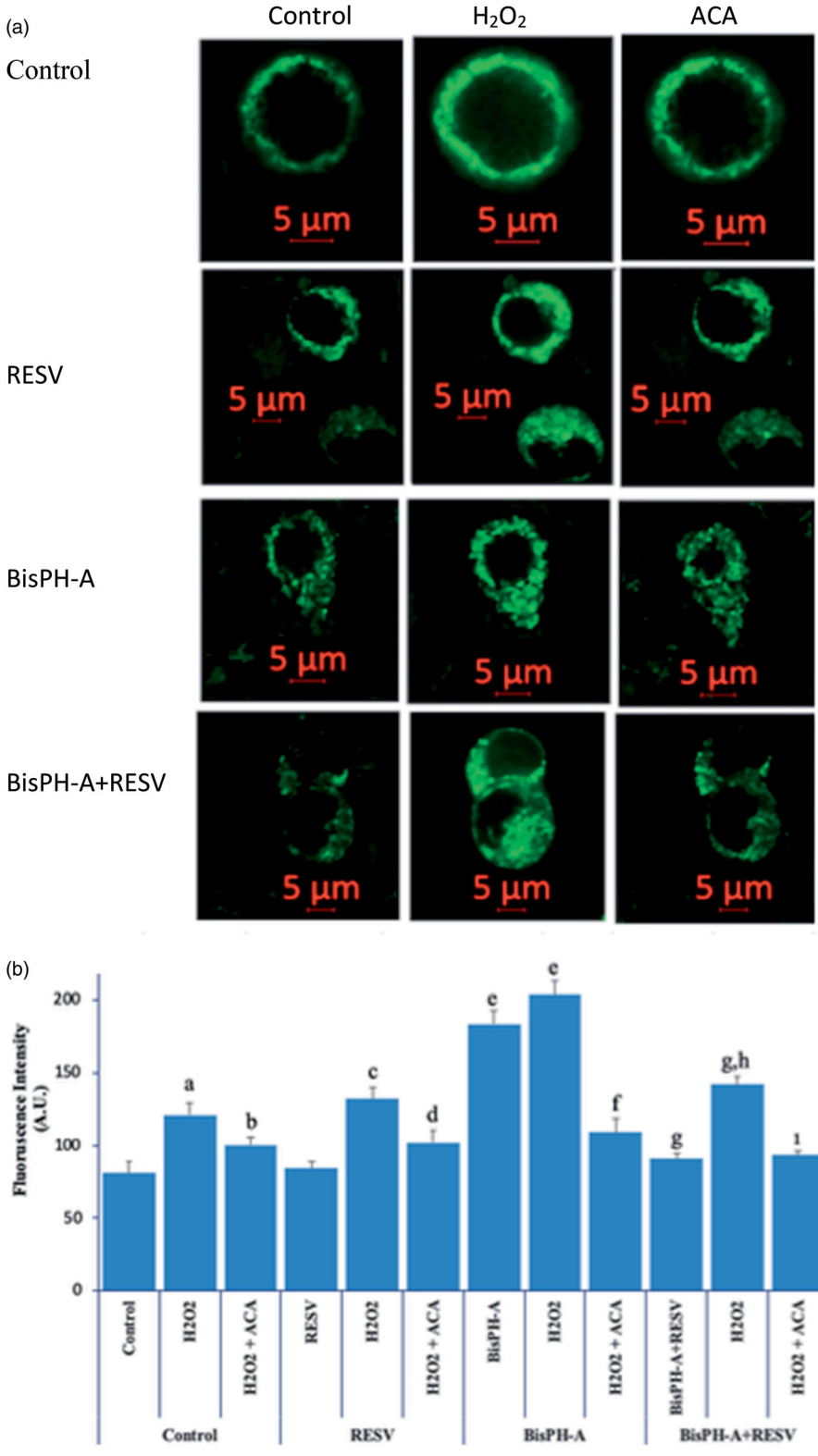

Figure 3. Effect of RESV (50 µM-24h) on the Ca^{2+} fluorescence intensity through TRPM2 activation in the BisPH-A induced mpkCDDcl$_{14}$ cells (mean ± SD and $n = 20$). The cells were stained with Fluo3 calcium dye and mean ± SD of fluorescence in 10 mm^2 of cell as arbitrary unit (A.U.) are presented; $n = 20$ independent experiments. The cells were extracellularly stimulated by H_2O_2 (100 µM for 15 min), but they were extracellularly inhibited by ACA (0,025 mM for 15 min) after washing with 1XPBS (a). The samples were analyzed by laser confocal microscopy fitted with zeiss 40 x oil immersion objective. Changes of the Ca^{2+} fluorescence intensity were shown by columns ($^a p \leq 0.01$ vs control group; $^b p \leq 0.05$ vs H_2O_2 group; $^c p \leq 0.01$ vs RESV group; $^d p \leq 0.05$ vs H_2O_2 group; $^e p \leq 0.001$ vs Control group; $^f p \leq 0.001$, $^g p \leq 0.001$ vs BisPH-A group; $^h p \leq 0.01$ vs BisPH-A + RESV group; $^i p \leq 0.01$ vs H_2O_2 group).

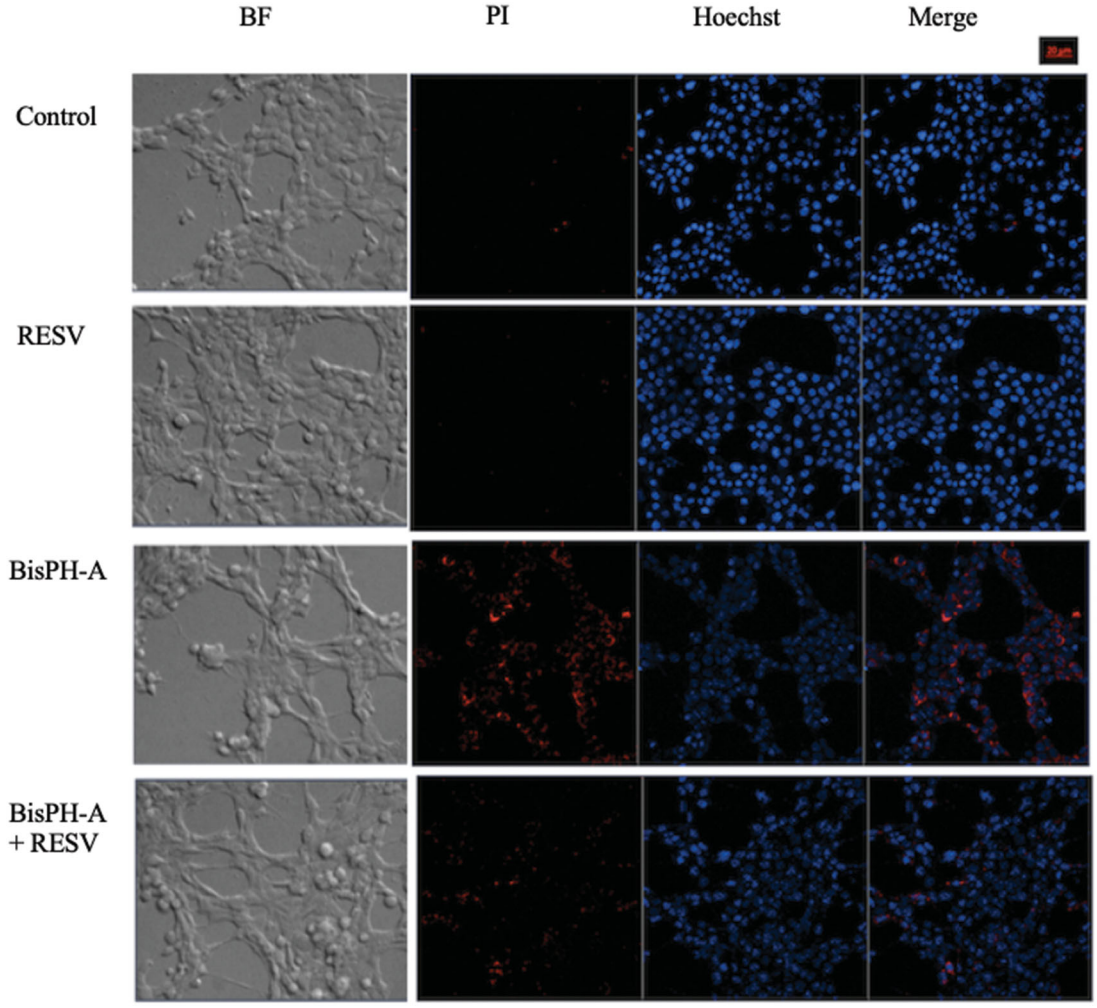

Figure 4. Exposure to BisPH-A induces cell death in mpkCDDcl$_{14}$ cells. Representative bright field and fluorescence images showing propidium iodide (PI), Hoechst staining and merge in control. Representative bright fild and fluorescence images showing PI and hoecst staining and merge in BisPH-A, RESV and BisPH-A + RESV groups.

BisPH-A-free on their thermal papers due to the increase in social awareness recently [45].

So what harmful effects does BisPH-A have after it enters our body? Scientific studies conducted include deformation of the intestinal structure of BisPH-A, especially the endocrine system and renal dysfunction, diabetes, liver and brain dysfunction, decreased sperm count, early sexual maturation in women, decreased sperm quality and infertility in men, obesity prevalence, immune deficiency and It even states that it causes numerous negative results until it reaches cancer cases. It is also stated that BisPH-A leaves the cell vulnerable to DNA damage [46,47]. It is especially worrying that the fetus in the womb is exposed to BisPH-A. As is known, BisPH-A can pass through the placenta and cross the blood brain barrier. Therefore, with the fetal circulatory system, all cells and especially the developing fetal brain are exposed to this substance during pregnancy. Animal studies show that perinatal BisPH-A exposure alters brain structure and development, induces early neurogenesis, and changes synaptic transmission and regulation [43,48].

The reason behind BisPH-A causing such negativity is that it disrupts the oxidant/antioxidant balance in the cells and eventually causes oxidative stress. Reactive oxygen metabolites such as superoxide anion and hydroxyl radicals are cytotoxic agents that oxidative damage by attacking membrane lipids, proteins and nucleic acids in cells [8]. These free radicals formed in cells and cytosolic reactive oxygen species (ROS) cause more cytosolic Ca^{2+} influx from outside the cell through special ion channels localized in the membrane. This results in a high $[Ca^{2+}]_i$ concentration. The high increase in this Ca^{2+}, along with mitochondrial membrane depolarization, even greater ROS increase, ATP depletion and eventually caspase cascades leading to apoptosis. Transient receptor potential melastatin 2 (TRPM2), which is a member of the melastatin subfamily of TRP channels, also plays a very important role in the regulation and homeostasis of Ca^{2+} influx through the plasma membrane. The sensitivity of TRPM2 to ROS, and in these cases to act as a sensor and increase intracellular expression levels, has shown that it may be important in therapeutic approaches. Oxidative stress

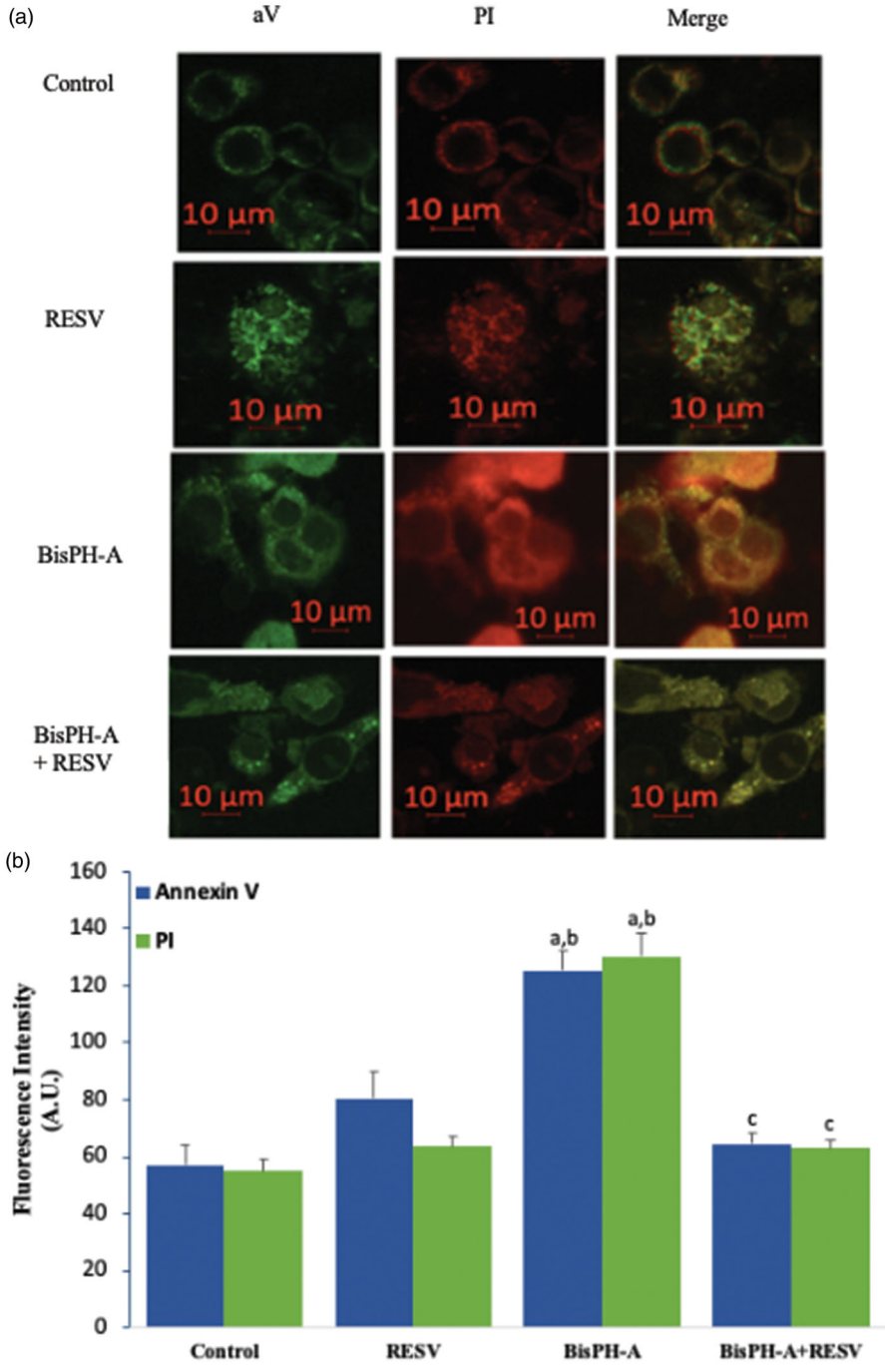

Figure 5. Effect of RESV on the apoptosis (Annexin V, aV and Propidium iodide, PI levels) in the BisPH-A induced mpkCDDcl$_{14}$ cells. The cells were incubated with BisPH-A(100 µM-24h) and RESV (50 µM-24h). Samples were analyzed by laser confocal microscopy fitted with zeiss 40x oil immersion objective. The mean ± SD of fluorescence per cell as arbitrary unit (A.U.) is presented; $n = 20$ independent experiments. (a) Representative images showing PI and annexin V-FITC staining in the mpkCDDcl$_{14}$ cells. Each panel consists of PI (red) and annexin V-FITC staining image (green). Scale bar is 10 µm. (b) Summary of the mean percentage of annexin V-FITC and PI under indicated conditions from 20 independent experiments. ($^{a}p \leq 0.001$ versus control groups; $^{b}p \leq 0.001$ versus RESV groups; $^{c}p \leq 0.001$ versus BisPH-A groups).

triggers ADPR by activation of poly (ADPR) polymerase-1 (PARP-1) [49]. ADPR is hydrolyzed to ribose-5-phosphate and AMP by NUDT9-H at the C terminal of TRPM2 channels and the channel is activated [50,51].

In this study, ACA and RESV significantly blocked channel activation from BispH-A in TRPM2 patch-clamp and calcium signal analysis (Figures 1, 2 and 3). In addition, TRPM2 blocker (ACA) while suppressing BisPH-A-induced ROS, mitochondrial membrane depolarization, caspase-3 & 9 and apoptosis values, significantly increased cell viability (Figures 7 and 8). However, we have also detected RESV effective suppression of BispH-A-induced apoptosis in confocal microscopy analysis using markers such as PI, Hoechst, Annexin V (Figures 4 and 5). We have shown here that mpkCCD$_{cl4}$ cells

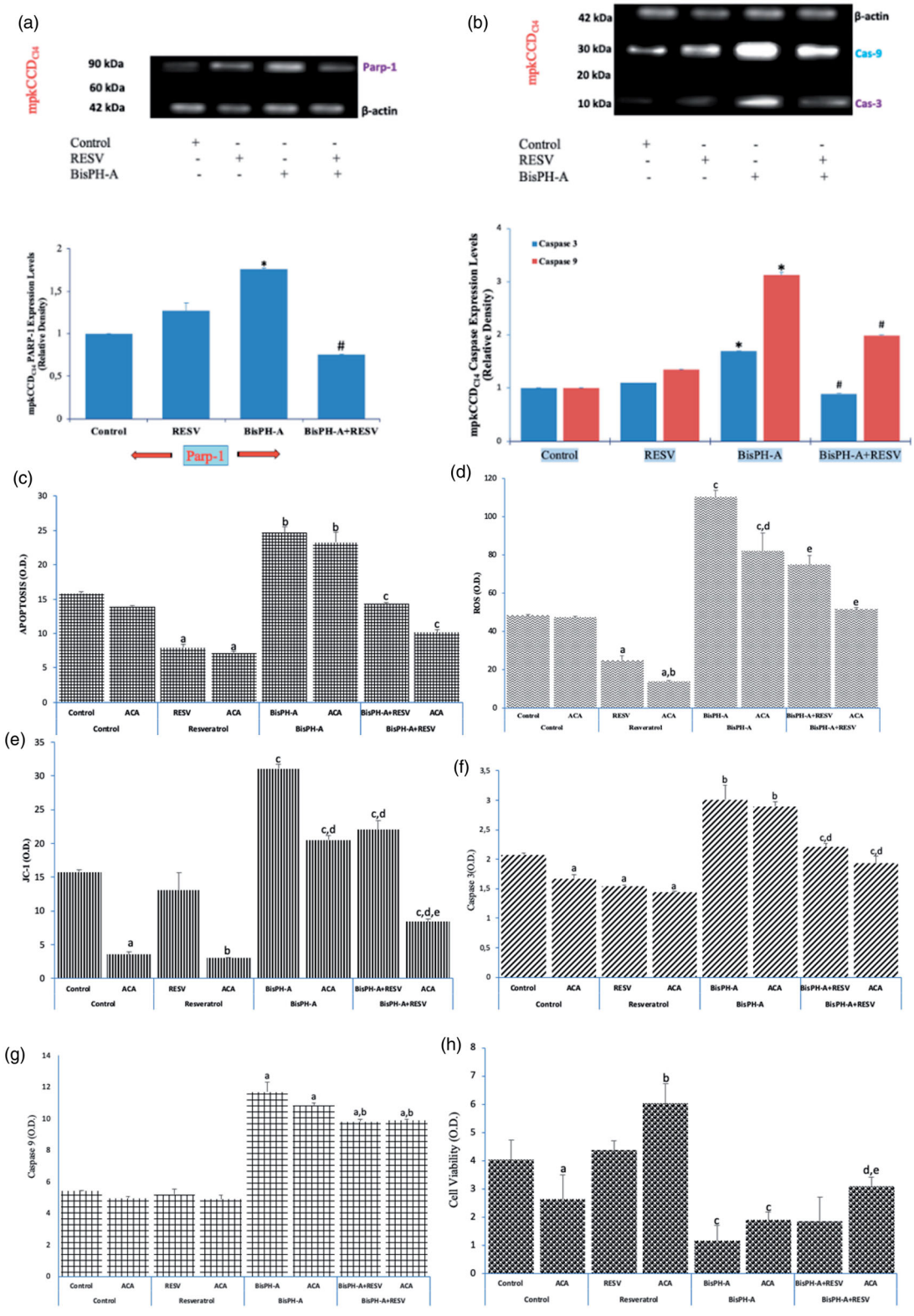

Figure 6. Effects of BisPH-A exposed and Resveratrol (RESV) treatment on PARP-1 (a) and caspase-3, 9 expression levels (b) in the mouse kidney cortical collecting duct cells ($mpkCCD_{cl4}$) (western blot analysis was used to examine the protein levels) (mean ± SD and $n = 3$). The values are presented as relative density (fold increase). ($^{*}p \leq 0.001$ vs control and $^{\#}p \leq 0.001$ vs BisPH-A groups). Effect of RESV (50 μM) treatment on the apoptosis levels (c) ($^{a}p \leq 0.001$ and $^{b}p \leq 0.001$ vs control; $^{c}p \leq 0.001$ vs BisPH-A), Reactive Oxygen Species (ROS) levels (d) ($^{a}p \leq 0.001$ vs control; $^{b}p \leq 0.01$ vs RESV; $^{c}p \leq 0.001$ vs control; $^{d}p \leq 0.001$, $^{e}p \leq 0.001$ vs BisPH-A), Mitochondrial Membrane Depolarization (JC-1) levels (e) ($^{a}p \leq 0.001$ vs control; $^{b}p \leq 0.01$ vs RESV; $^{c}p \leq 0.001$ vs control; $^{d}p \leq 0.001$vs BisPH-A;, $^{e}p \leq 0.001$ vs BisPH-A + RESV), Caspase-3 levels (f) ($^{a}p \leq 0.05$ vs control; $^{b}p \leq 0.001$ vs Control and RESV; $^{c}p \leq 0.001$ vs RESV; $^{d}p \leq 0.001$vs BisPH-A), Caspase-9 levels (g) ($^{a}p \leq 0.001$ vs control; $^{b}p \leq 0.05$ vs BisPH-A) and cell viability (MTT) levels (h) ($^{a}p \leq 0.05$ vs control; $^{b}p \leq 0.01$ vs RESV; $^{c}p \leq 0.001$ vs control and RESV; $^{d}p \leq 0.05$ vs BisPH-A;, $^{e}p \leq 0.05$ vs BisPH-A + RESV) in the Bisphenol A (BisPH-A) exposed $mpkCCD_{cl4}$ cells (mean ± SD and $n = 3$). The cells were stimulated by Hydrogen Peroxide (H_2O_2 and 100 μM for 2 min.) but they were inhibited by Anthranilic Acid (ACA 25 μM for 10 min).

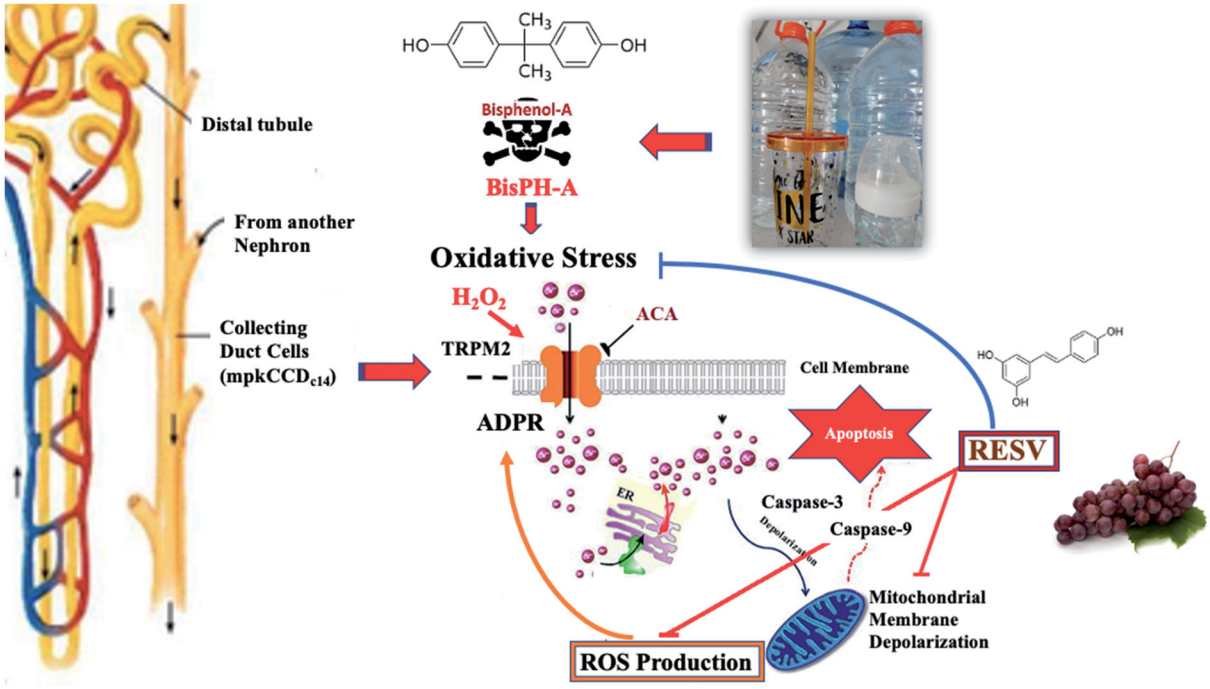

Figure 7. Protective effect of Resveratrol in bispH-A induced TRPM2 overactivation in mouse kidney cortical collecting duct (mpkCCD$_{cl4}$) cells.

are involved in BisPH-A induced TRPM2 overactivity and mitochondrial ROS production and triggering of apoptotic cascades.

A powerful anti-inflammatory, antioxidant Resveratrol (3,4′,5-trihydroxystilbene), which is a naturally found phenolic in grape peel and kernel, has been reported to clear free oxygen radicals, reduce oxidative stress and increase cell viability by regulating mitochondrial function [52–54]. It is reported that this cytoprotective activity of resveratrol is achieved by stimulating the endogenous antioxidant system, providing control over the genes where apoptotic pathways are managed, and direct scavenging of ROS [55,56]. In terms of these antioxidant properties, RESV has always attracted attention. We have demonstrated here that RESV has a great protective effect on the oxidative damage caused by BisPH-A in kidney collection canal cells. We have also demonstrated that the underlying molecular mechanisms can be inhibition of TRPM2 and mitochondria-induced oxidative stress pathways. We estimate that RESV, integrated into cellular membranes, effectively reduces BisPH-A-induced cytotoxic effects such as intracellular Ca^{2+} overload, caspase activations and ROS production. As far as we know so far, no studies have been conducted in mpkCCDcl4 cells where BisPH-A-induced oxidative stress is addressed in terms of TRPM2 channels and RESV. When we look at mitochondrial membrane depolarization, ROS, apoptosis levels and caspase activities, we can see that these values increased with BisPH-A normalize with RESV. However, increased ROS in the environment further increased the levels of intracellular free calcium, further depolarizing the mitochondria and accelerated the process that would drag the cell into apoptosis. We observed the contribution of TRPM2 to this intracellular calcium elevation at electrophysiological and Fura-2 calcium signal analysis using appropriate agonist (ADPR, H2O2) and suitable antagonists (ACA) in BisPH-A groups. RESV acted as an inhibitor of TRPM2 channels, suppressing the rise of calcium in the cell. RESV kept both levels of intracellular calcium at normal values, both by clearing cytosolic ROS and by inhibiting TRPM2 channels, and saved cells from going to apoptosis.

However, thousands of basic science experiments in in-vitro and animal models show that resveratrol has many positive effects. For example, RESV has been reported to inhibit the oxidation of low-density lipoproteins, thereby preventing atherosclerosis [57,58]. Studies have shown that RESV significantly increases superoxide dismutase (SOD) activity and reduces ROS production. RESV is known to act as a cleanser of hydroxyl, superoxide and other radicals. Thus, it prevents DNA lesions and lipid peroxidation in cell membranes. As an antioxidant, Resveratrol has two effects: it can increase the activity of antioxidant enzymes and act as a free radical scavenger. RESV has been shown to maintain the concentration of intracellular antioxidants in biological systems. Resveratrol has also been reported to increase the concentration of certain antioxidant enzymes, such as glutathione peroxidase, glutathione reductase [11–13].

PARP-1 has been reported to play a major role in ROS-mediated TRPM2 gate and cell death [15]. In this study, up-regulation of BispH-A-induced apoptotic pathways, PARP1 activity, TRPM2 channel activity in mpkCCD$_{cl4}$ cells was reversed by complete inhibition of these channels and oxidative stress parameters with RESV.

Acknowledgements

The results of this study were presented as posters at 7th Oxidative Stress, Calcium Signaling and TRP Channel World Congress 20-23 April 2018 Alanya, Antalya, Turkey http://2018.cmos.org.tr

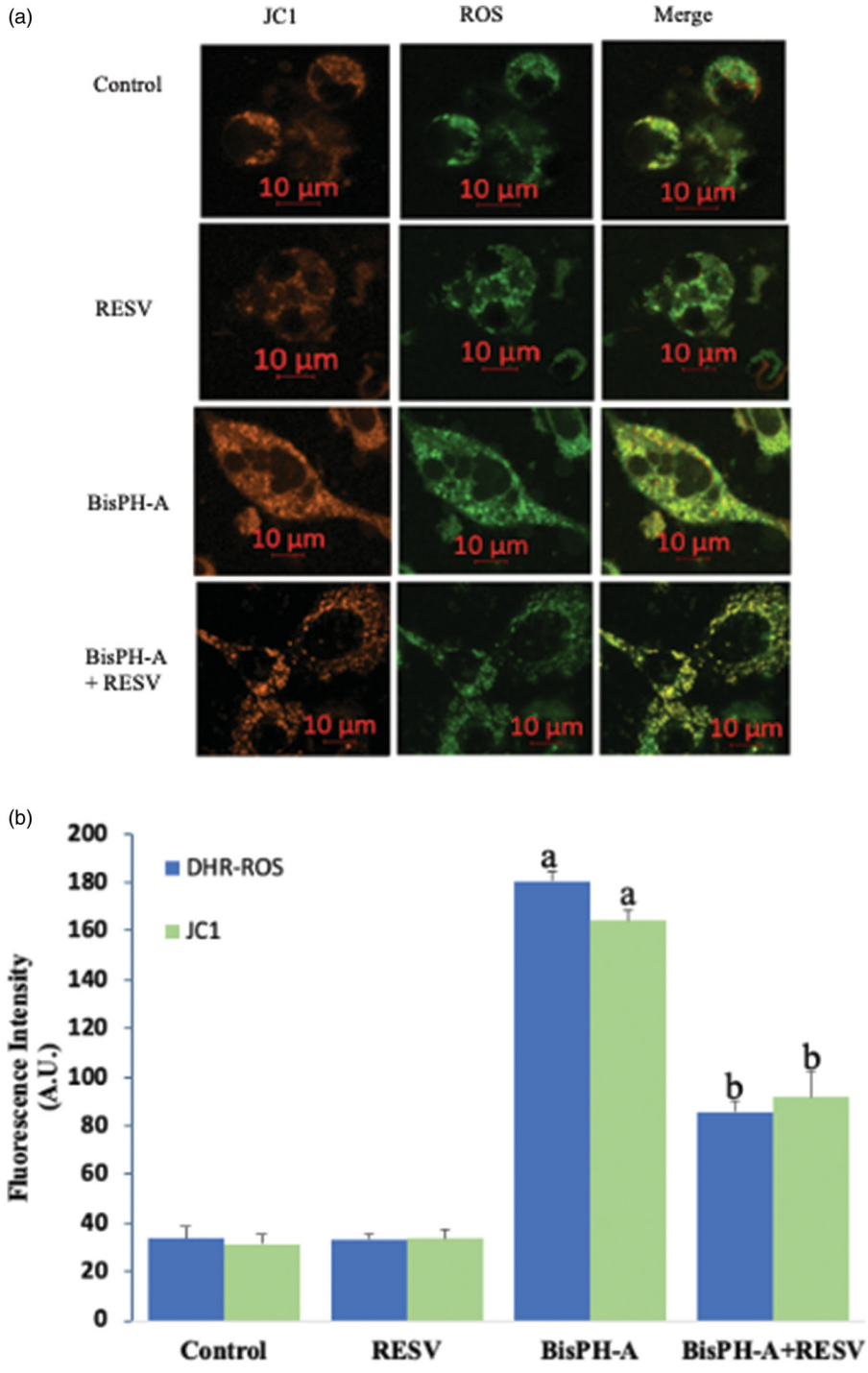

Figure 8. Bisphenol-A (BisPH-A) increases the mitochondrial membrane depolarization (JC1) and intracellular reactive oxygen species (ROS) production levels of mpkCCD$_{c14}$ cells. Resveratrol (RESV) treatment prevents the increase in these values. The cells were incubated by BisPH-A (100 µM for 24 h) and RESV (50 µM for 24 h). Samples were analyzed by laser confocal microscopy fitted with zeiss 40x oil immersion objective. The mean ± SD of fluorescence per cell as arbitrary unit (A.U.) is presented ($n = 10$ independent experiments). Representative images showing JC1 and DHR-123 staining in the mpkCCD$_{c14}$ cells. Each panel contains JC1-staining image (brown) and DHR-123 (green). Scale bar is 10 µm. (b) Summary of the mean percentage of JC1 and DHR-123 under indicated conditions from 10 independent experiments. $^{*} \leq 0.001$ vs. control groups, # ≤ 0.001 vs. BisPH-A groups.

Disclosure statement

The authors declare that there are no conflicts of interest.

ORCID

Bilal Çiğ https://orcid.org/0000-0001-7832-066X
Kenan Yildizhan http://orcid.org/0000-0002-6585-4010

References

[1] Ashby J, Tinwell H. Uterotrophic activity of bisphenol A in the immature rat. Environ. Health Perspect. 1998;106(11): 719–720.
[2] Kimber I. Bisphenol A and immunotoxic potential: A commentary. Regul. Toxicol. Pharmacol. 2017;90:358–363.
[3] Gassman NR. Induction of oxidative stress by bisphenol A and its pleiotropic effects. Environ. Mol. Mutagen. 2017;58(2):60–71.

[4] Wang H, Ding Z, Shi QM, et al. Anti-androgenic mechanisms of Bisphenol A involve androgen receptor signaling pathway. Toxicology. 2017b;387:10–16.
[5] Hoekstra EJ, Simoneau C. Release of bisphenol A from polycarbonate: a review. Crit Rev Food Sci Nutr. 2013;53(4):386–402.
[6] Makris KC, Andra SS, Jia A, et al. Association between water consumption from polycarbonate containers and bisphenol A intake during harsh environmental conditions in summer. Environ. Sci. Technol. 2013;47(7):3333–3343.
[7] Chung YH, Han JH, Lee SB, et al. Inhalation toxicity of bisphenol A and its effect on estrous cycle, spatial learning, and memory in rats upon whole-body exposure. Toxicol Res. 2017;33(2): 165–171.
[8] Hatef A, Zare A, Alavi SM, et al. Modulations in androgen and estrogen mediating genes and testicular response in male goldfish exposed to bisphenol A. Environ. Toxicol. Chem. 2012;31(9): 2069–2077.
[9] Ma Y, Liu H, Wu J, et al. The adverse health effects of bisphenol A and related toxicity mechanisms. Environ. Res. 2019;176: 108575.
[10] Michalowicz J. Bisphenol A–sources, toxicity and biotransformation. Environ Toxicol Pharmacol. 2014;37:738–758.
[11] Di Donato M, Cernera G, Giovannelli P, et al. Recent advances on bisphenol-A and endocrine disruptor effects on human prostate cancer. Mol Cell Endocrinol. 2017;457:35–42.
[12] Murata M, Kang JH. Bisphenol A (BPA) and cell signaling pathways. Biotechnol. Adv. 2018;36(1):311–327.
[13] Elswefy SE, Abdallah FR, Atteia HH, et al. Inflammation, oxidative stress and apoptosis cascade implications in bisphenol A-induced liver fibrosis in male rats. Int J Exp Pathol. 2016;97(5):369–379.
[14] Wang C, Qi S, Liu C, et al. Mitochondrial dysfunction and Ca^{2+} overload in injured sertoli cells exposed to bisphenol A. Environ. Toxicol. 2017a;32(3):823–831.
[15] Orrenius S, Gogvadze V, Zhivotovsky B. Calcium and mitochondria in the regulation of cell death. Biochem. Biophys. Res. Commun. 2015;460(1):72–81.
[16] Bootman MD, Berridge MJ, Roderick HL. Calcium signalling: more messengers, more channels, more complexity. Curr Biol. 2002; 12(16):R563–565.
[17] Orrenius S, Zhivotovsky B, Nicotera P. Regulation of cell death: the calcium-apoptosis link. Nat. Rev. Mol. Cell Biol. 2003;4(7): 552–565.
[18] Kumar B, Iqbal MA, Singh RK, et al. Resveratrol inhibits TIGAR to promote ROS induced apoptosis and autophagy. Biochimie. 2015; 118:26–35.
[19] Xia N, Daiber A, Forstermann U, et al. Antioxidant effects of resveratrol in the cardiovascular system. Br. J. Pharmacol. 2017; 174(12):1633–1646.
[20] Gurer-Orhan H, Ince E, Konyar D, et al. The role of oxidative stress modulators in breast cancer. Curr Med Chem. 2018;25(33): 4084–4101.
[21] Liu C, Montell C. Forcing open TRP channels: Mechanical gating as a unifying activation mechanism. Biochem Biophys Res Commun. 2015;460(1):22–25.
[22] Naziroglu M, Cig B, Ozgul C. Neuroprotection induced by N-acetylcysteine against cytosolic glutathione depletion-induced Ca2+ influx in dorsal root ganglion neurons of mice: role of TRPV1 channels. Neuroscience. 2013;242:151–160.
[23] Balaban H, Nazıroğlu M, Demirci K, et al. The protective role of selenium on scopolamine-induced memory impairment, oxidative stress, and apoptosis in aged rats: the involvement of TRPM2 and TRPV1 channels. Mol Neurobiol. 2017;54(4):2852–2868.
[24] Nazıroğlu M, Çiğ B, Yazğan Y, et al. Albumin evokes Ca^{2+}-induced cell oxidative stress and apoptosis through TRPM2 channel in renal collecting duct cells reduced by curcumin. Sci Rep. 2019; 9(1):12403.
[25] Naylor J, Al-Shawaf E, McKeown L, et al. TRPC5 channel sensitivities to antioxidants and hydroxylated stilbenes. J Biol Chem. 2011;286(7):5078–5086.
[26] Kaya O, Kaptaner B. Antioxidant defense system parameters in isolated fish hepatocytes exposed to bisphenol A – effect of vitamin C. Acta Biol Hung 2016;67(3):225–235.
[27] Akpinar H, Naziroglu M, Ovey IS, et al. The neuroprotective action of dexmedetomidine on apoptosis, calcium entry and oxidative stress in cerebral ischemia-induced rats: Contribution of TRPM2 and TRPV1 channels. Sci Rep. 2016;6:37196.
[28] Grynkiewicz G, Poenie M, Tsien RY. A new generation of Ca2+ indicators with greatly improved fluorescence properties. J Biol Chem 1985;260(6):3440–3450.
[29] Espino J, Bejarano I, Paredes SD, et al. Protective effect of melatonin against human leukocyte apoptosis induced by intracellular calcium overload: relation with its antioxidant actions. J Pineal Res. 2011;51(2):195–206.
[30] Nazıroğlu M, Çiğ B, Blum W, et al. Targeting breast cancer cells by MRS1477, a positive allosteric modulator of TRPV1 channels. PLoS One. 2017;12(6):e0179950.
[31] Naziroglu M, Demirdas A. Psychiatric disorders and TRP channels: focus on psychotropic drugs. CN. 2015;13(2):248–257.
[32] Nazıroğlu M, Sahin M, Ciğ B, et al. Hypericum perforatum modulates apoptosis and calcium mobilization through voltage-gated and TRPM2 calcium channels in neutrophil of patients with Behcet's disease. J Membr Biol. 2014;247(3):253–262.
[33] Keil VC, Funke F, Zeug A, et al. Ratiometric high-resolution imaging of JC-1 fluorescence reveals the subcellular heterogeneity of astrocytic mitochondria. Pflugers Arch. 2011;462(5):693–708.
[34] Uslusoy F, Nazıroğlu M, Çiğ B. Inhibition of the TRPM2 and TRPV1 channels through hypericum perforatum in sciatic nerve injury-induced rats demonstrates their key role in apoptosis and mitochondrial oxidative stress of sciatic nerve and dorsal root ganglion. Front Physiol. 2017;8:335.
[35] Gobira PH, Lima IV, Batista LA, et al. N-arachidonoyl-serotonin, a dual FAAH and TRPV1 blocker, inhibits the retrieval of contextual fear memory: Role of the cannabinoid CB1 receptor in the dorsal hippocampus. J. Psychopharmacol. (Oxford). 2017;31(6):750–756.
[36] Özdemir ÜS, Nazıroğlu M, Şenol N, et al. Hypericum perforatum attenuates spinal cord injury-induced oxidative stress and apoptosis in the dorsal root ganglion of rats: involvement of TRPM2 and TRPV1 channels. Mol Neurobiol. 2016;53(6):3540–3551.
[37] Deveci HA, Akyuva Y, Nur G, et al. Alpha lipoic acid attenuates hypoxia-induced apoptosis, inflammation and mitochondrial oxidative stress via inhibition of TRPA1 channel in human glioblastoma cell line. Biomed Pharmacother. 2019;111:292–304.
[38] An X, Fu Z, Mai C, et al. Increasing the TRPM2 channel expression in human neuroblastoma SH-SY5Y cells augments the susceptibility to ROS-induced cell death. Cells 8. 2019;8(1):28.
[39] Ertilav K, Nazıroğlu M, Ataizi ZS, et al. Selenium enhances the apoptotic efficacy of docetaxel through activation of TRPM2 channel in DBTRG glioblastoma cells. Neurotox Res. 2019;35(4): 797–808.
[40] Zhao Z, Qu W, Wang K, et al. Bisphenol A inhibits mucin 2 secretion in intestinal goblet cells through mitochondrial dysfunction and oxidative stress. Biomed Pharmacother. 2019;111:901–908. Mar
[41] Joshi DC, Bakowska JC. Determination of mitochondrial membrane potential and reactive oxygen species in live rat cortical neurons. J Vis Exp. 2011;(51):2704:1–4.
[42] Zhang Y, Xiao Y, Ma Y, et al. ROS-mediated miR-21-5p regulates the proliferation and apoptosis of Cr(VI)-exposed L02 hepatocytes via targeting PDCD4. Ecotoxicol Environ Saf. 2020;191:110160.
[43] Grohs MN, Reynolds JE, Liu J, APrON Study Team, et al. Prenatal maternal and childhood bisphenol a exposure and brain structure and behavior of young children. Environ Health. 2019;18(1):85.
[44] LaKind JS, Naiman DQ. Temporal trends in bisphenol A exposure in the United States from 2003-2012 and factors associated with BPA exposure: Spot samples and urine dilution complicate data interpretation. Environ. Res. 2015;142:84–95. Oct
[45] Pivnenko K, Pedersen GA, Eriksson E, et al. Bisphenol A and its structural analogues in household waste paper. Waste Manag. 2015;44:39–47.

[46] Oliveira KJ, Chiamolera MI, Giannocco G, et al. Thyroid function disruptors: from nature to chemicals. J Mol Endocrinol. 2018; 62(1):R1–R19.
[47] Porreca I, Ulloa-Severino L, Almeida P, et al. Molecular targets of developmental exposure to bisphenol A in diabesity: a focus on endoderm-derived organs. Obes Rev. 2017;18(1):99–108.
[48] Xu X, Liu X, Zhang Q, et al. Sex-specific effects of bisphenol-A on memory and synaptic structural modification in hippocampus of adult mice. Horm Behav. 2013;63(5):766–775.
[49] Ataizi ZS, Ertilav K, Nazıroğlu M. Mitochondrial oxidative stress-induced brain and hippocampus apoptosis decrease through modulation of caspase activity, Ca2+ influx and inflammatory cytokine molecular pathways in the docetaxel-treated mice by melatonin and selenium treatments. Metab Brain Dis. 2019;34(4): 1077–1089.
[50] Zhu T, Zhao Y, Hu H, et al. TRPM2 channel regulates cytokines production in astrocytes and aggravates brain disorder during lipopolysaccharide-induced endotoxin sepsis. Int Immunopharmacol. 2019;75:105836.
[51] Roberge S, Roussel J, Andersson DC, et al. TNF-α-mediated caspase-8 activation induces ROS production and TRPM2 activation in adult ventricular myocytes. Cardiovasc Res. 2014;103(1):90–99.
[52] Neal SE, Buehne KL, Besley NA, et al. Resveratrol protects against hydroquinone-induced oxidative threat in retinal pigment epithelial cells. Invest Ophthalmol Vis Sci. 2020;61(4):329.
[53] Ghosh AK, Rao VR, Wisniewski VJ, et al. Differential activation of glioprotective intracellular signaling pathways in primary optic nerve head astrocytes after treatment with different classes of antioxidants. Antioxidants (Basel). 2020;9(4):324.
[54] Ahmed H, Jahan S, Ullah H, et al. The addition of resveratrol in tris citric acid extender ameliorates post-thaw quality parameters, antioxidant enzymes levels, and fertilizing capability of buffalo (Bubalus bubalis) bull spermatozoa. Theriogenology. 2020;152: 106–113.
[55] Kong AN, Yu R, Hebbar V, et al. Signal transduction events elicited by cancer prevention compounds. Mutat Res. 2001;480–481: 231–241.
[56] Chen CY, Jang JH, Li MH, Surh YJ. Resveratrol upregulates heme oxygenase-1 expression via activation of NF-E2-related factor 2 in PC12 cells. Biochem Biophys Res Commun. 2005;331(4):993–1000.
[57] Akbari M, Tamtaji OR, Lankarani KB, et al. The effects of resveratrol on lipid profiles and liver enzymes in patients with metabolic syndrome and related disorders: a systematic review and meta-analysis of randomized controlled trials. Lipids Health Dis. 2020; 19(1):25.
[58] Hong M, Li J, Li S, et al. Resveratrol derivative, trans-3, 5, 4′-trimethoxystilbene, prevents the developing of atherosclerotic lesions and attenuates cholesterol accumulation in macrophage foam cells. Mol Nutr Food Res. 2020;64(6): e1901115.